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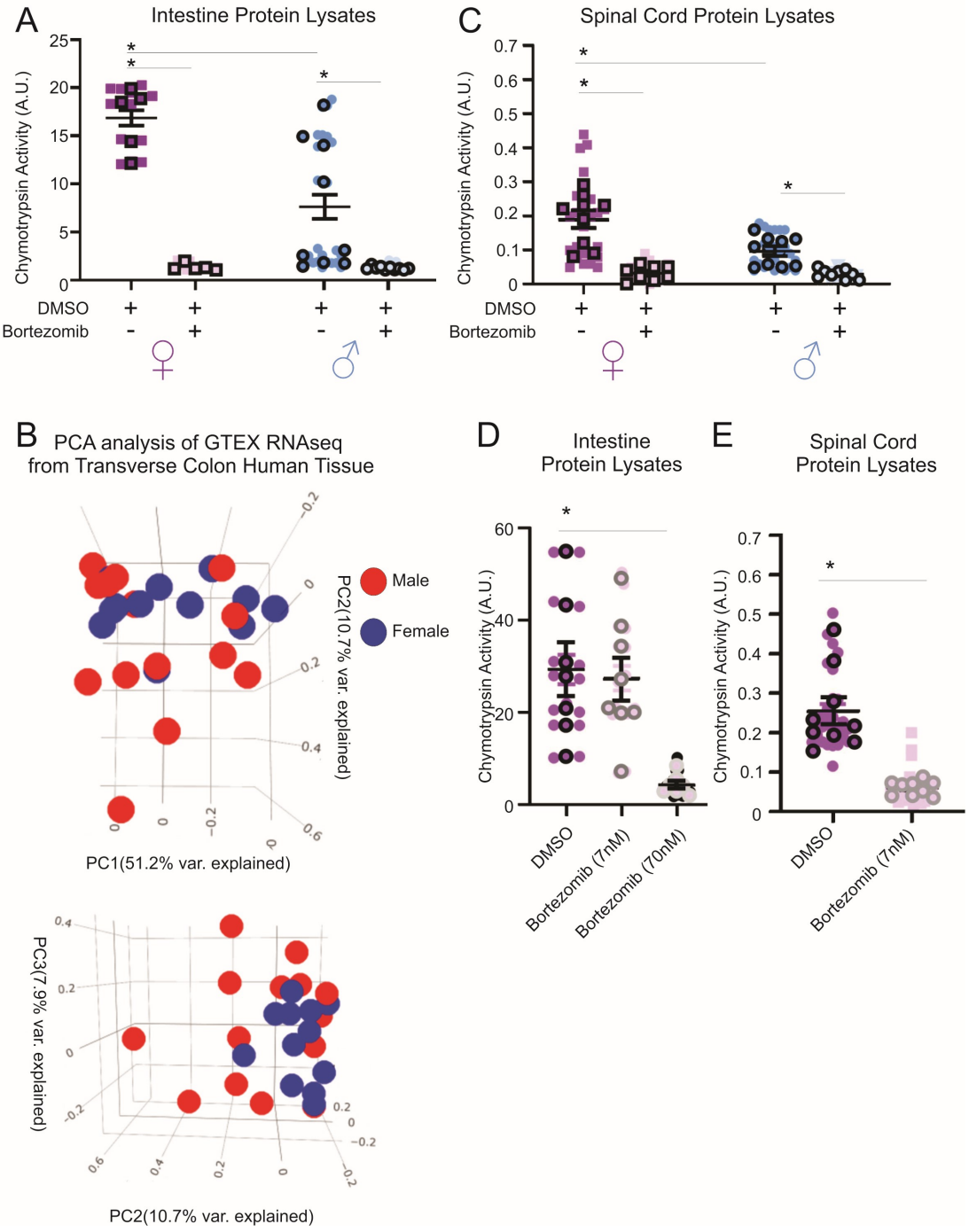
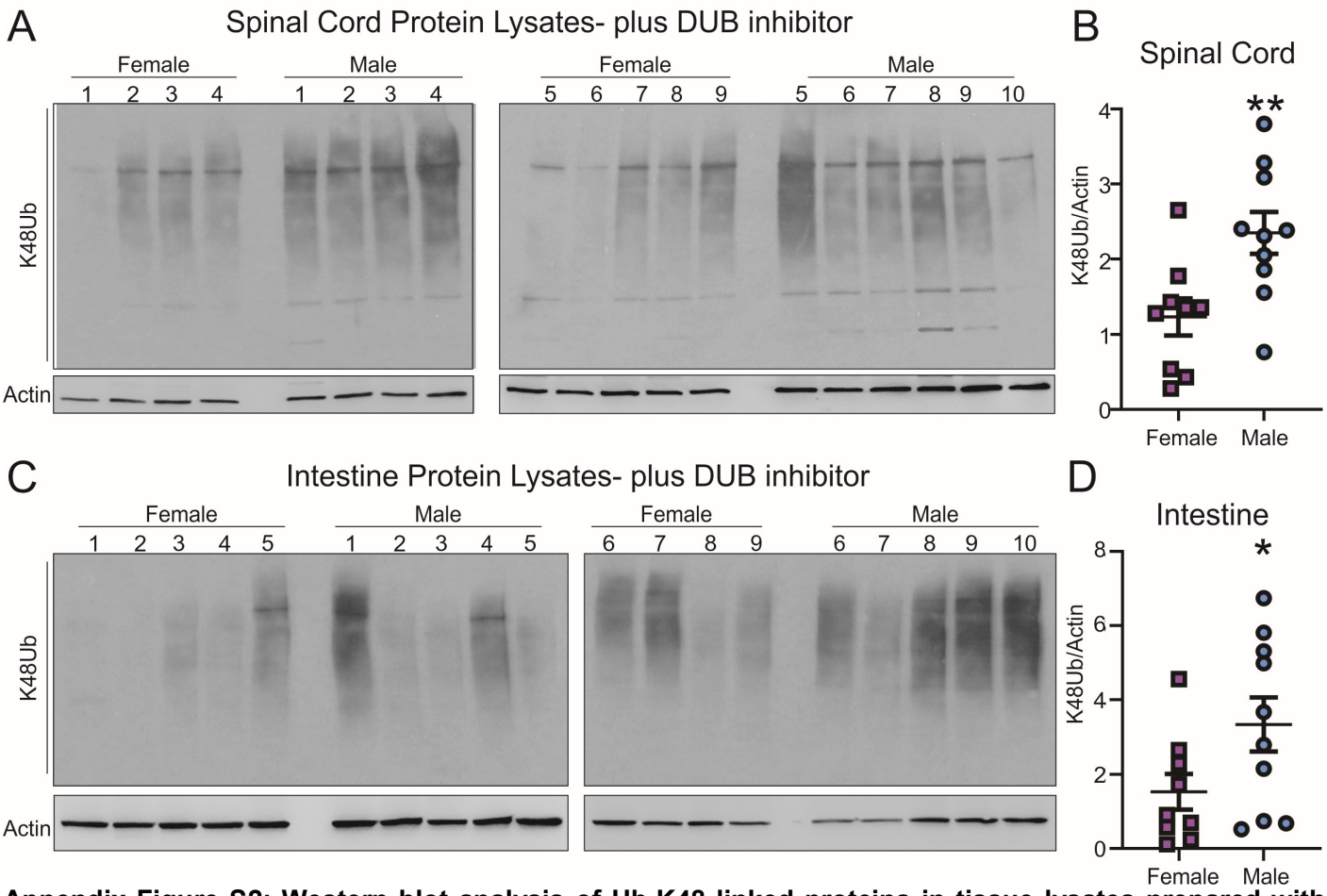
Appendix Figure S1



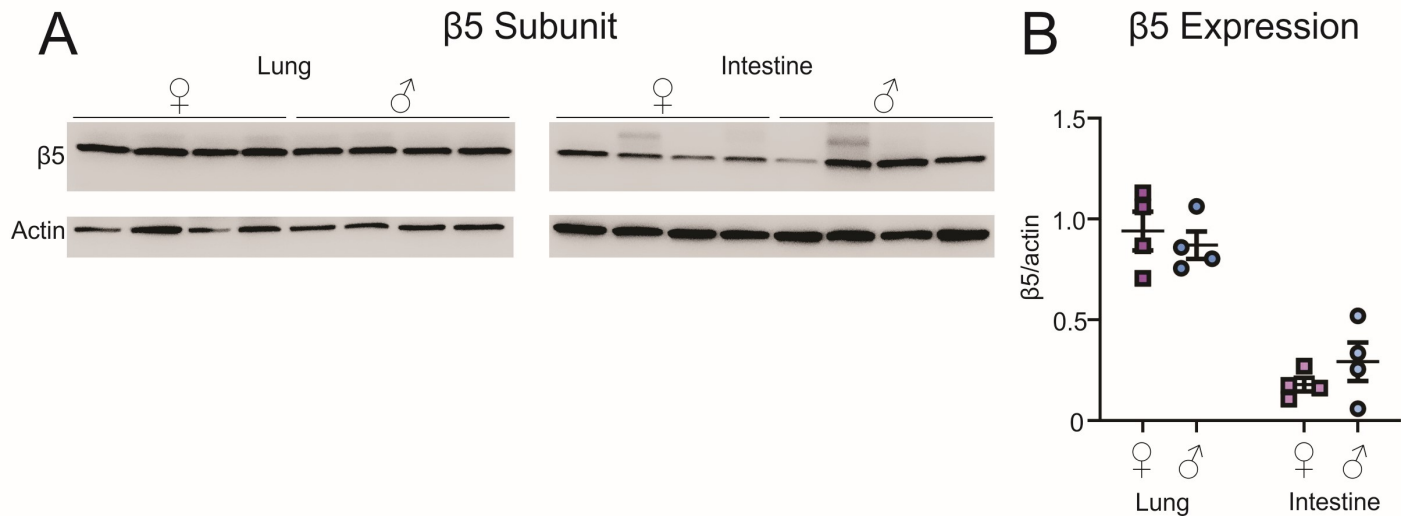
Appendix Figure S1: Bortezomib eliminates chymotrypsin-like activity of the proteasome in protein lysates. (A) Intestine protein lysate was incubated with 2mM Bortezomib or an equivalent volume of DMSO at 4°C for 1 hour before measuring chymotrypsin-like proteasome activity with Bortezomib at 50μM. (B) Principal component analysis of GTEX RNAseq data from human female (n=12) and male (n=14) transverse colon. (C) Spinal cord protein lysate was incubated with 2mM Bortezomib or an equivalent volume of DMSO at 4°C for 1 hour before measuring chymotrypsin-like proteasome activity with Bortezomib at 50μM. Bordered points indicate biological replicates and un-bordered points indicate technical replicates.
* indicates $p<0.05$ by two-tailed student's t-test. (D-E) Intestine (D) or spinal cord (E) protein lysate was incubated with the indicated concentrations of Bortezomib. A, C, D, E Each central line and error bar indicate mean +/- SEM of a biological replicates.

Appendix Figure S2



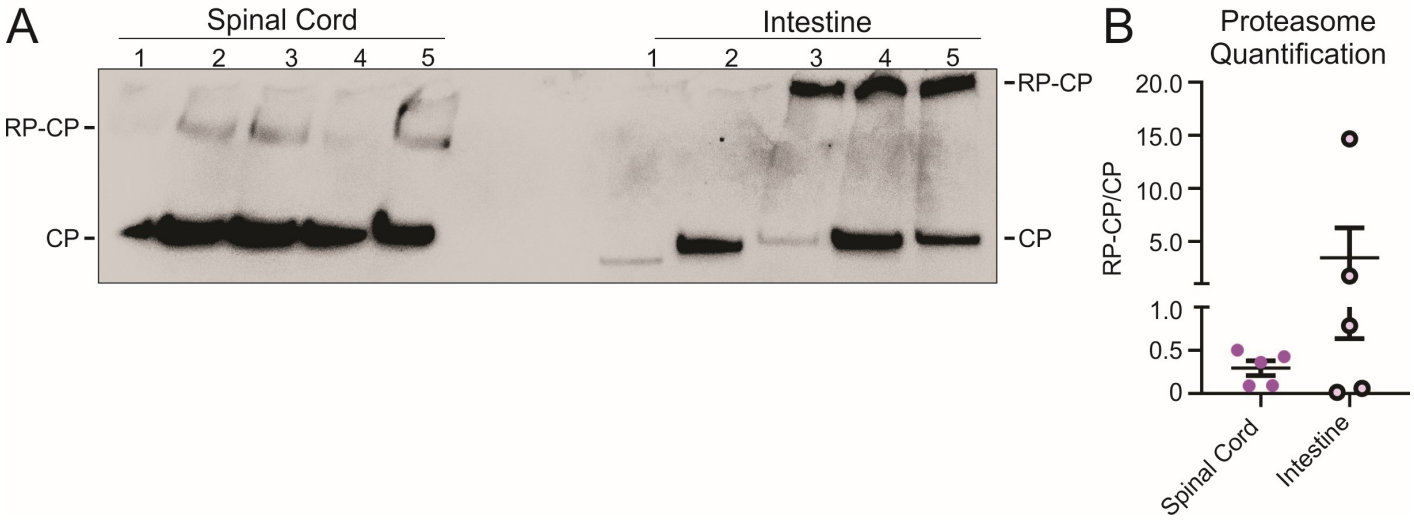
Appendix Figure S2: Western blot analysis of Ub-K48 linked proteins in tissue lysates prepared with DUB inhibition. (A) Spinal cord tissue lysates were prepared in the presences of the DUB inhibitor PR-619 and analyzed by western blot for UB-K48. (B) Quantification of panel A. (C) Intestine tissue lysates were prepared in the presences of the DUB inhibitor PR-619 and analyzed by western blot for UB-K48. (D) Quantification of panel C. * indicates $p<0.05$. ** indicates $p<0.005$ by two-tailed student's t-test. B, D Each central line and error bar indicate mean +/- SEM of a biological replicates (n=9 females and n=10 males).

Appendix Figure S3



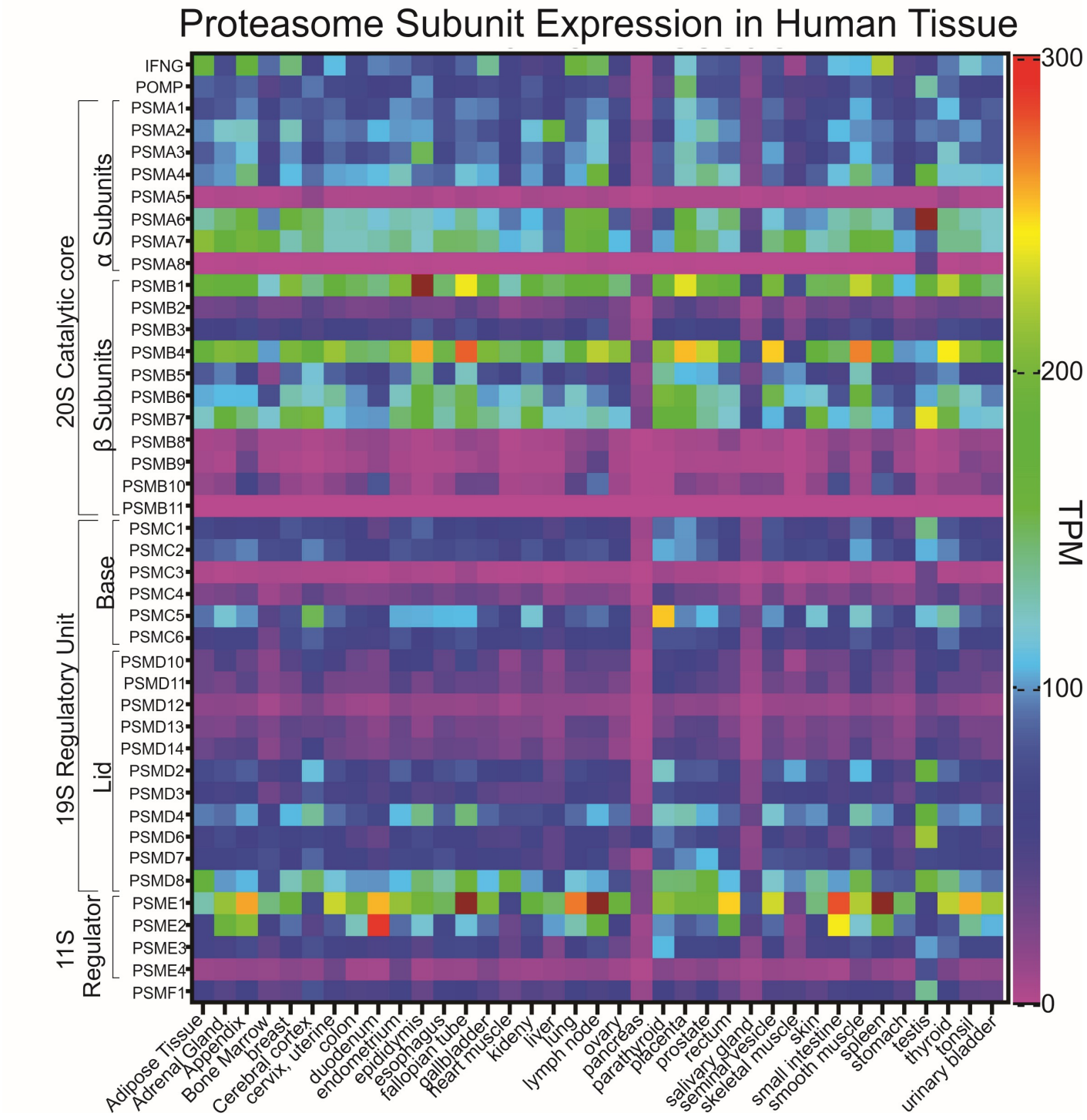
Appendix Figure S3: **No correlation between β5 subunit protein and observed proteasome activity.** A) Western blot analysis of the β5 subunit of the 20S proteasome in lung (tissue with low measured chymotrypsin-like proteasome activity) and intestine (tissue with high measured chymotrypsin-like proteasome activity) protein lysate from female and male mice. B) Quantification of panel C. Each central line and error bar indicate mean +/- SEM of a biological replicates (n=4 females and n=4 males).

Appendix Figure S4



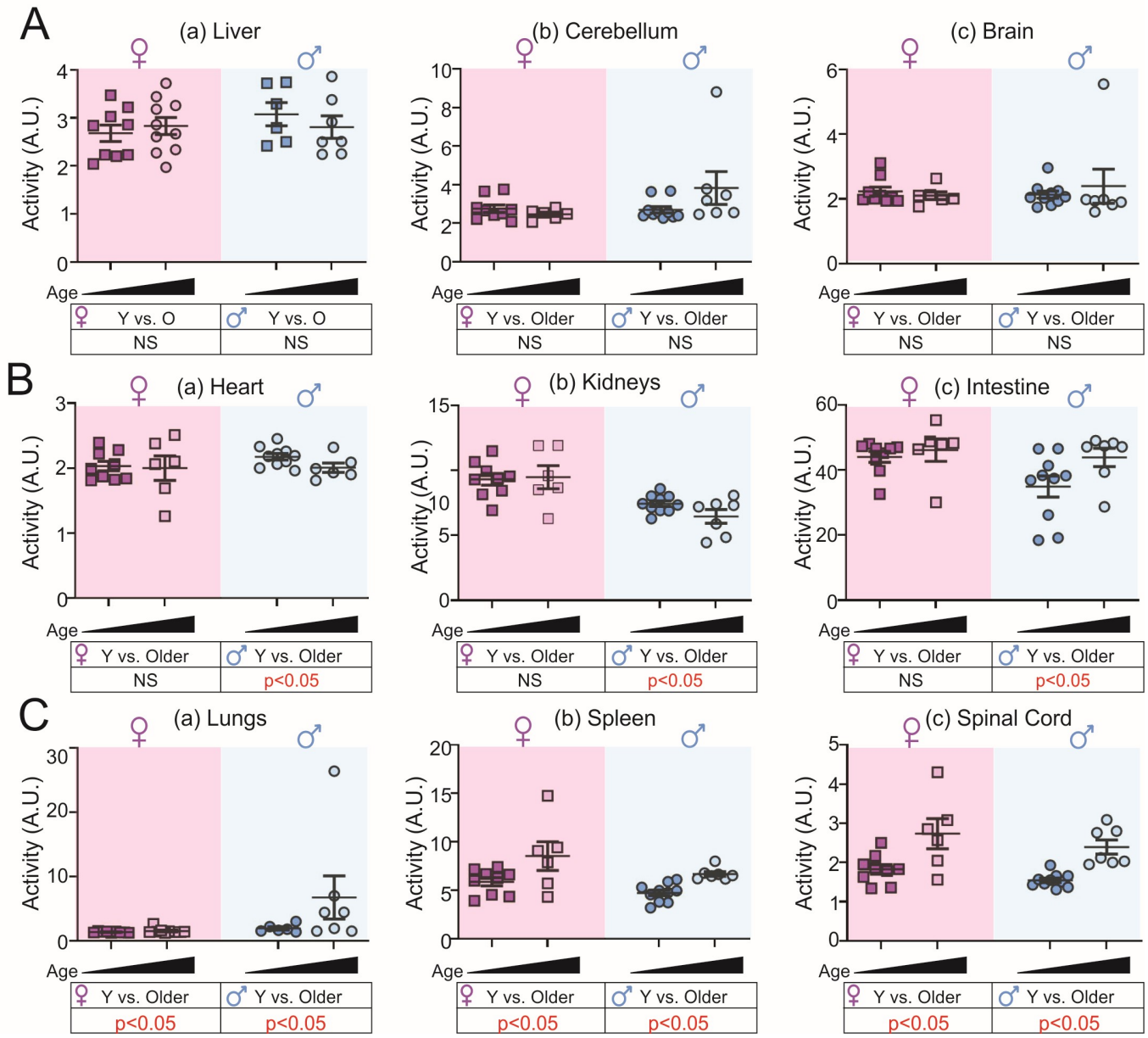
Appendix Figure S4: Proteasome Assembly differs between spinal cord and intestine. (A) Native gel followed by western of β5 subunit to distinguish unassembled catalytic core (CP) or assembled with regulatory core (RP) in female (n=5) mice in spinal cord and intestine. B) Quantification of A, Each central line and error bar indicate mean +/- SEM of a biological replicates (n=5 per group).

Appendix Figure S5



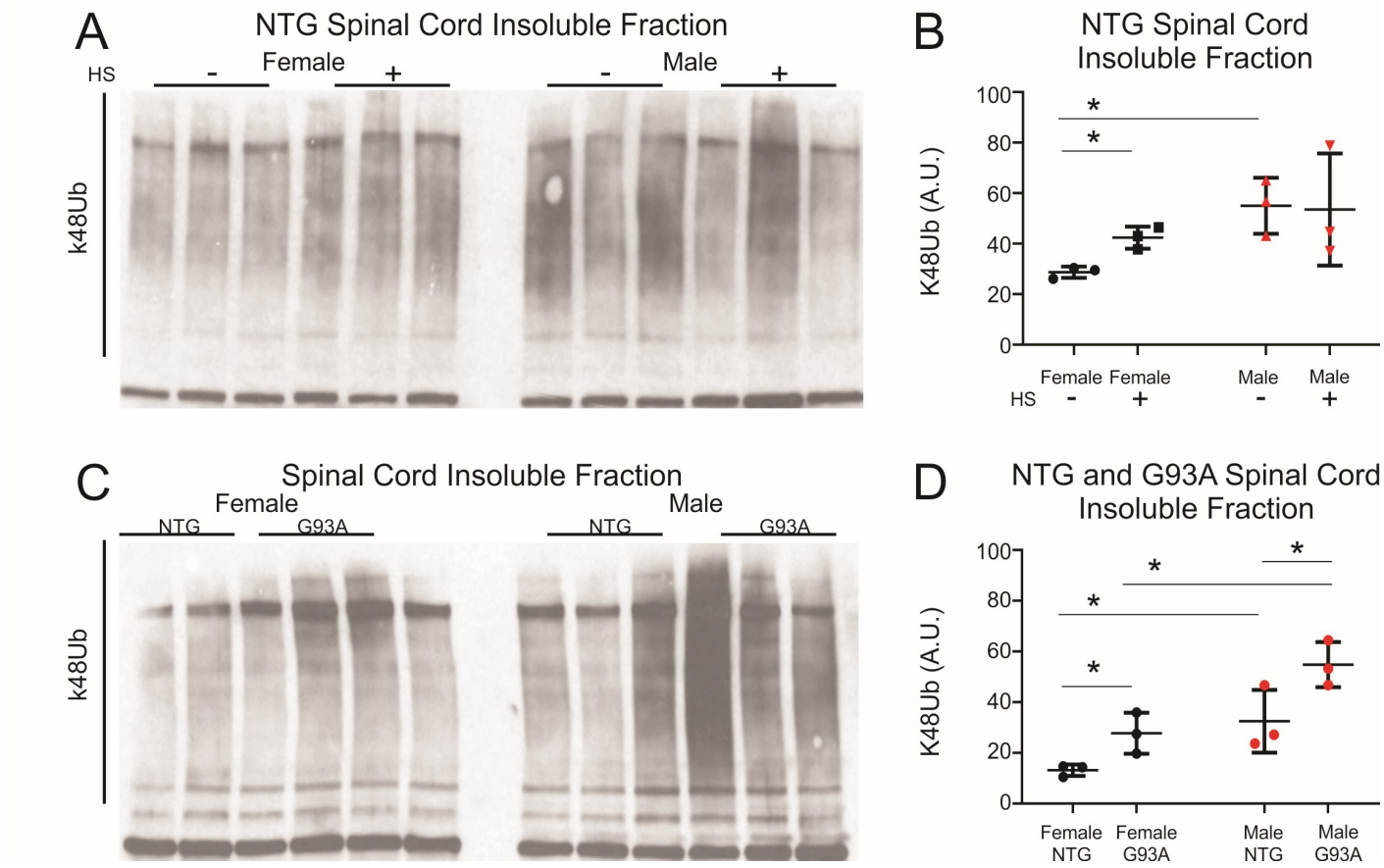
Appendix Figure S5: Proteasome subunit expression varies across tissue. (A) Heat map of RNAseq data from the Human Protein Atlas project across 37 tissues.

Appendix Figure S6



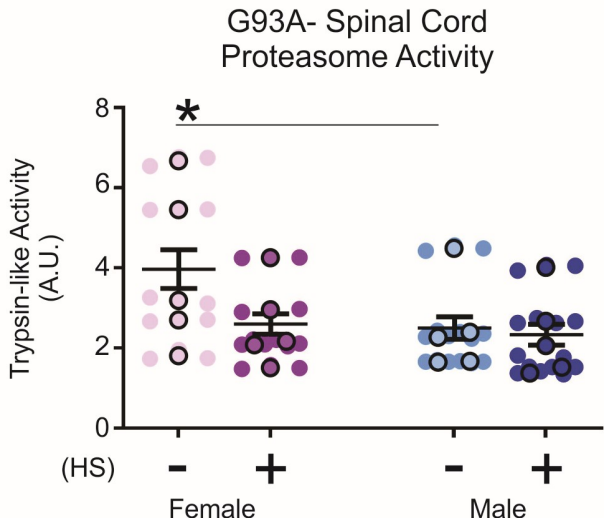
Appendix Figure S6: **Trypsin-like proteasome activity shows differential decline between tissues and sexes.** Trypsin-like activity in protein lysates from mice that where either "young" (3-6 months old, female (n=9) and male (n=10)) or "older" (10-15 months old, female (n=6) and male (n=7)) at the time of tissue collection. Tissues either did not show significant decline (A), declined significantly in males only (B), or both females and males (C). Each data point indicates a single mouse performed in triplicate. Each central line and error bar indicate mean +/- SEM of a biological replicates (n=9 young females, n=6 older females, n=10 young males, n=7 older males). P values obtained by unpaired two-tailed student's t-test comparing young and older samples are indicated.

Appendix Figure S7



Appendix Figure S7: **K48Ub proteins in G93A and non-transgenic (NTG) control mice.** (A) Western blot analysis of UB-K48 protein in the insoluble fraction of spinal cord protein lysates from female and male NTG mice with or without heat shock (B) quantification of panel A. (C) Western blot analysis of UB-K48 protein in the insoluble fraction of female and male G93a and NTG mice. (D) Quantification of panel C. * indicates $p<0.05$ by two-tailed student's t-test. B, D . Each central line and error bar indicate mean +/- SEM of a biological replicates (n=3 females and n=3 males).

Appendix Figure S8



Appendix Figure S8: Heat shock decreases trypsin-like proteasome activity in female G93A mice. Trypsin-like activity in the spinal cord of G93A mice plus or minus heat shock was recorded. Bordered points indicate biological replicates (n=5 per group) and un-bordered points indicate technical replicates.
* indicates $p<0.05$ by two-tailed student's t-test. Each central line and error bar indicate mean +/- SEM of a biological replicates.